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Morphological and molecular support for *Amphithrax verrucosus* (H. Milne Edwards, 1832) and *Amphithrax aculeatus* (Herbst, 1790) (Crustacea, Decapoda, Brachyura) as valid species

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Abstract

The large degree of morphological variations, particularly amongst juveniles, has led to inconsistencies in the literature regarding the taxonomic status of *Amphithrax aculeatus* (Herbst, 1790) and *Amphithrax verrucosus* (H. Milne Edwards, 1832). As a result of recent biodiversity sampling initiatives in Barbados, West Indies, multiple specimens of *Amphithrax aculeatus* and *A. verrucosus* have been collected. This has prompted us to undertake a thorough reassessment of their morphological and molecular characteristics. Moreover, morphological differences in the carapace, antennae, chelipeds, pereopods and the male first gonopod (G1) supports *A. aculeatus* and *A. verrucosus* as separate species. Molecular phylogenetic analysis, based on newly-generated sequences of the 12S rRNA, 16S rRNA and ITS-1 genes also shows that *A. verrucosus* is a separate species and sister taxa to *A. aculeatus*. The total number of species within the genus *Amphithrax* is now brought to eleven. However, our molecular analysis also shows that the taxonomic placement of *Amphithrax armatus* (Saussure, 1853) within *Amphithrax* is questionable.

Key Words

Amphithrax, 12S, 16S, ITS-1, Lesser Antilles, Mithracidae

Introduction

The superfamily Majoidea Samouelle, 1819, consists of over 950 species of ecologically and economically important brachyuran crabs (Calado et al. 2003; Guiomar et al. 2007; Hultgren and Stachowicz 2008; Santana et al. 2016). Within this superfamily, the hairy clinging crabs of the genus *Amphithrax* are found dwelling amongst coral rocks and rubble, under sea anemones and in rock crevices in the intertidal zones to subtidal zones of up to 60 m depth (Baeza et al. 2010). They are found in the tropics and subtropical regions (Windsor and Felder 2017) and are especially abundant in nearshore rubble and shallow-water (0–4 m depth) marine habitats of Barbados (Parasram et al. 2023). Current taxonomy supports ten species in *Amphithrax* Windsor & Felder, 2017, nine of which have an Amphiamerican distribution and one, *Amphithrax caboverdianus* (Türkay, 1986), which is found in the eastern Atlantic. However, taxonomic disparity exists within the literature regarding the identity of hairy clinging crabs and this is largely due to the variability in size, shape, larval and adult forms within this genus. In that regard, the relationship and taxonomic status of *Amphithrax aculeatus* (Herbst, 1790) and its presently considered junior subjective synonym, *Amphithrax verrucosus* (H. Milne Edwards, 1832) remains controversial.

Amphithrax aculeatus was originally described as *Cancer aculeatus* by Herbst in 1790. Later, in 1816,

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Latreille described the genus *Mithrax* and designated *Cancer aculeatus* Herbst, 1790 as its type species. After that, H. Milne Edwards, in 1832, described *Mithrax verrucosus*, based on syntypes collected from the Antilles, specifically from Martinique. Subsequent research by Desbonne and Schramm (1867) identified both *M. aculeatus* and *M. verrucosus* in samples collected in Guadeloupe. This period also saw the establishment of *Mithrax trispinosus* Kingsley, 1879 from Florida, albeit based on an exceptionally small specimen. The type for this species was later reported as lost by Rathbun (1925). Further, Rathbun, in 1892, described *Mithrax pilosus* from specimens collected in the Bahamas and, in a subsequent study in 1901, described *Mithrax plumosus* from Puerto Rico.

The early 20th century was marked by Rathbun's taxonomic revisions, which had a significant influence on the genus's classification. In her 1901 work, she proposed a synonymy between *M. pilosus* and *M. aculeatus*, treating the latter as a junior synonym of the former. Furthermore, in her 1925 work, she designated both *M. trispinosus* and *M. plumosus* as junior synonyms of *M. verrucosus*. However, Rathbun provided no explanation for these taxonomic changes.

The latter half of the 20th century saw a continuation of these taxonomic debates. Researchers such as Williams (1984) included *Mithrax verrucosus* in their findings for Florida. In parallel, Powers (1977), Abele and Kim (1986) and Wagner (1990) asserted the validity of both *M. pilosus* and *M. verrucosus*. Wagner's study stood out for emphasising the ontogenetic shifts in morphology and he highlighted that juveniles of the two species were morphologically more similar than their adult counterparts.

Ng et al. (2008: 123) corrected the precedence of *Mithrax aculeatus* over *M. pilosus* set by Rathbun, stating that the lectotype specimen of *Cancer aculeatus* Herbst, 1790, is probably a conspecific of *M. pilosus*. They further clarified the synonymic relationship between *C. aculeatus* Fabricius, 1793 and *C. aculeatus* Herbst, 1790 and retained both *Mithrax aculeatus* and *M. verrucosus* as valid species.

More recently, molecular approaches to taxonomy have become central to species delineation. Based on molecular evidence from three mitochondrial (12S, 16S, COI) and two nuclear genes (18S, H3), Windsor and Felder (2014) placed *M. verrucosus* as a junior synonym of *M. aculeatus*. Later, Windsor and Felder (2017) made some amendments to their previous work (Windsor and Felder 2014) and established the now accepted genus *Amphithrax* Windsor & Felder, 2017 to accommodate several species belonging to *Mithrax* Latrielle, 1816, including *Amphithrax aculeatus* (Herbst, 1790) and *Amphithrax verrucosus* (H. Milne Edwards, 1832). Windsor and Felder (2014) reported that the carapace shape and texture in large specimens of *A. verrucosus* closely matches the illustration of *C. aculeatus* by Herbst (1790, pl. 19, fig. 104).

In more recent studies, Carmona-Suárez and Poupin (2016) and Poupin (2018) have revisited the classification of *A. aculeatus* and *A. verrucosus*. Based on morphological characters, they proposed the separate classification of *A. aculeatus* and *A. verrucosus*, arguing for both species to retain their distinct status, a rationale followed by Parasram et al. (2023) in their brachyuran crabs' inventory of Barbados. Thus, the present study attempts to resolve the taxonomic status and phylogenetic relationship between *A. aculeatus* and *A. verrucosus* by using an integrative approach that incorporates both morphological and new molecular evidence.

Materials and methods

Sample collection and preservation

A total of sixty-two (62) specimens of *Amphithrax aculeatus* (24) and *A. verrucosus* (38) were collected from nearshore rubble and shallow subtidal habitats (~ 4 m depth) on the west and south coasts of the island of Barbados, West Indies (Fig. 1A, B). They were collected by hand, with the aid of hand nets and with cage crab traps (Fig. 1C). Collected specimens were transported to the laboratory, euthanised by freezing, and preserved in 70% ethanol. Specimens collected during this study are stored at the Barbados Laboratory of Systematic Zoology (BLSZ). A stereomicroscope (Olympus SZ7, Model #: SZ2-ILST) was used to examine specimens and fresh (after briefly freezing) and preserved specimen images were taken with a Nikon D3300 DSLR camera equipped with a 55 mm super macro lens and with a Toupcam full HD microscope camera (Model #: XCZM, Series HDMI 1080 P) mounted on the Olympus SZ7 microscope. Measurements of the carapace width (CW) and carapace length (CL) were taken with a vernier caliper with an accuracy of 0.01 mm.

Taxonomic classification

Information regarding the synonym, geographic distribution, material examined, and general remarks are included for each species. The morphological terminology follows that of Rathbun (1925) and Davie et al. (2015a). Taxonomic classification mostly follows that of Ng et al. (2008) and Davie et al. (2015b), but Guinot (1967), Manning and Chace (1990), Guinot and Tavares (2003) and Windsor and Felder (2014, 2017) were also considered.

Additional abbreviations used in the text are: **CW** = carapace width (measured dorsally at the widest point of the carapace, including lateral spines); **CL** = carapace length (measured from the bottom of the rostral sinus to the posterior margin of the carapace). Measurements for some studied specimens were not taken; **Idem** = The aforementioned locality; ♂ = male; ♀ = female; **juv.** = juveniles; **ovig.** = ovigerous females; **G1** = male first gonopod; **cm** = centimetre; **mm** = millimetre; **m** = metre; **fig./figs** = figure/s; **tab.** = table; **vol.** = volume; **BI** = Bayesian Inference; **ML** = Maximum Likelihood;

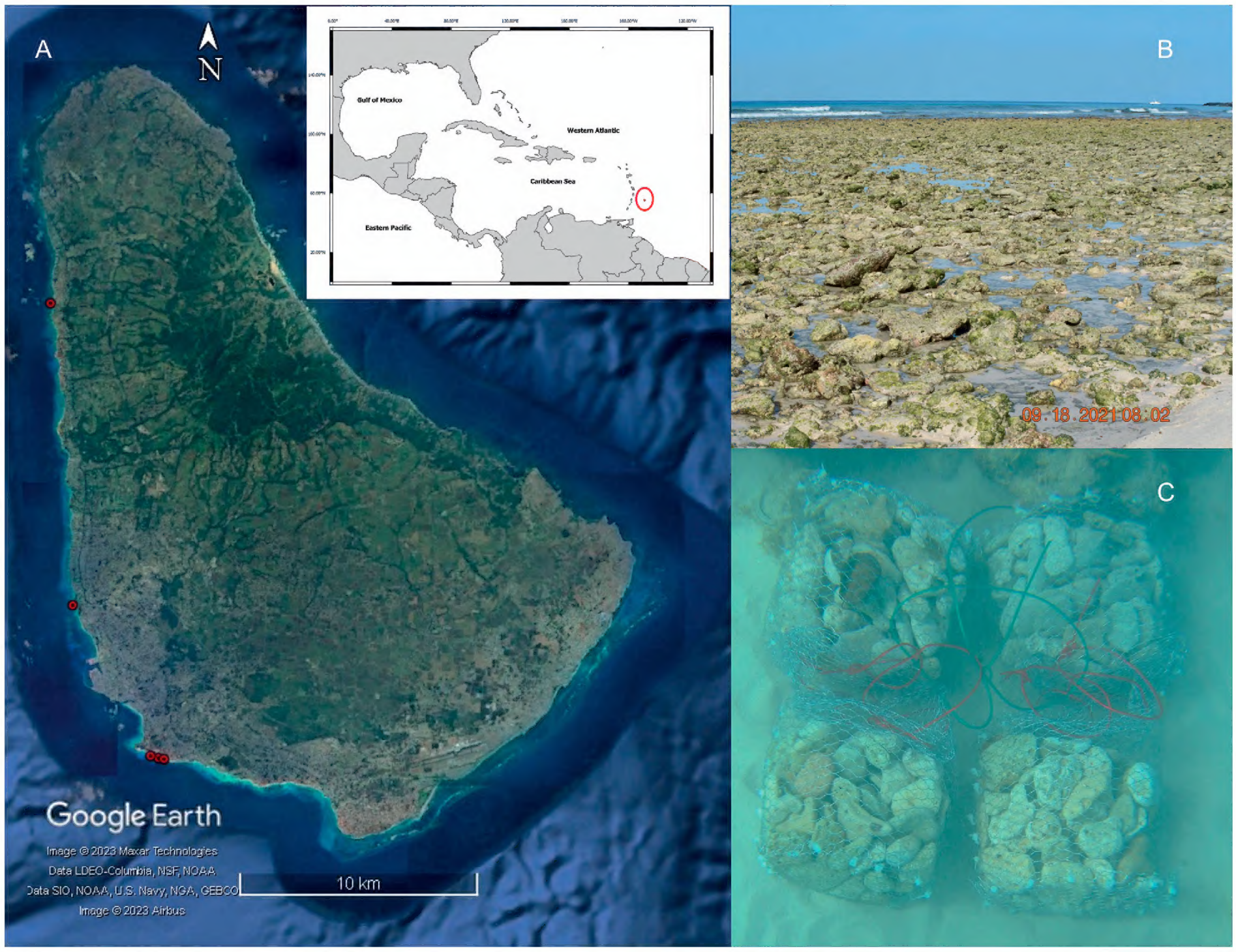

Figure 1. A. Map of Barbados with sampling locations during this study and position of Barbados (red circle) within the Caribbean region; **B.** Nearshore rubble habitat (exposed at low tide); **C.** A cluster of cage crab traps in subtidal habitat. Photos: Nadeshinie Parasram.

SEM = Scanning Electron Microscope; **Pp** = Posterior Probability. Specimens examined are deposited in the Barbados Laboratory of Systematic Zoology, University of the West Indies, Barbados (**BLSZ**); Coleção de Crustáceos do Departmento de Biologia – Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto, Universidade de São Paulo, Brazil (**CCDB**); Grupo de Investigación en Carcinología, Escuela de Ciencias Aplicadas de Mar, Núcleo Nueva Esparta, Universidade de Oriente (**GIC**); Museum of Comparative Zoology, Harvard University (**MCZ**); Muséum national d'Histoire naturelle, France (**MNHN**); Museu de Zoologia da Universidade de São Paulo, Brazil (**MZUSP**); National Museum of Marine Biology and Aquarium, Taiwan (**NMMBA**); University of Louisiana at Lafayette Zoological Collection, USA (**ULLZ**); National Museum of Natural History, Smithsonian Institution, USA (**USNM**); Museum für Naturkunde Berlin, Germany (**ZMB**).

DNA extraction, PCR, and sequencing

Muscle tissue was extracted from the ambulatory legs and chelipeds from 10 specimens each of *A. aculeatus* and *A. verrucosus* and total genomic DNA was extracted from the fresh muscle tissue using the ZYMO Quick-DNA Miniprep DNA extraction kits (catalogue # D3025), following the manufacturer's instructions. The tissue lysis stage was modified to incubate the muscle tissue in Proteinase K for one hour instead of the three hours suggested by the protocol, as this provided better results. Partial sequences of the two mitochondrial (12S rRNA, 16S rRNA) and one nuclear (ITS-1) genes were amplified with the following primers 12Sai/12Sbi (Palumbi et al. 1991), 16SF/16SR (Hultgren and Stachowicz 2008), and SP-1-3'/SP-1-5'138 (Chu et al. 2001).

Polymerised chain reactions (PCR) were carried out in 25 µl volumes with concentrations as follows: 12.5 µl (2X) Master Mix (Applied Biosystem), 0.5 µl (10 µM) of each primer, 6.5 µl of nuclease free water and 5 µl of template DNA. PCR amplifications for each gene were as follows: for 12S, initial denaturation of 2 min, followed by 35 cycles of denaturation for 30 s at 94 °C, annealing for 30 s at 55 °C, extension for 1 min at 72 °C and a final extension for 7 min at 72 °C; for 16S, initial denaturation of 2 min, followed by 35 cycles of denaturation 30 s at 94 °C, annealing for 30 s at 52 °C, extension for 1 min at 72 °C and a final extension for 5 min at 72 °C; and

for ITS-1, initial denaturation of 3 min, followed by 35 cycles of denaturation for 0.15 s at 94 °C, annealing for 0.45 s at 57 °C, extension for 1 min at 72 °C and a final extension for 10 min at 72 °C. Amplified PCR products were visualised on 2% agarose gels and PCR amplicons were sent to Macrogen (Seoul, Republic of Korea) for bi-directional sequencing.

Phylogenetic analysis

Sequences obtained by this study for *A. aculeatus* and *A. verrucosus* were combined with those from Baeza et al. (2010), Windsor and Felder (2014) and Assugeni et al. (2016) which are available on GenBank. Locality information and GenBank accession numbers for all taxa included in the molecular analysis are provided in Table 1. The sequences obtained by our study were checked for quality and trimmed of both forward and reverse primers using the programme BioEdit v.7.2.5. Consensus sequences were generated in Geneious Prime 2023.0.4 (https://www.geneious.com/). Multiple sequence alignment was performed on individual datasets for each gene (12S, 16S and ITS-1) using the MAFFT FFT-NS-1 (Katoh and Standley 2013) alignment algorithm and final alignments were checked manually for presence of incongruence and/or gaps.

Alignments for the 12S, 16S and ITS-1 genes were concatenated in Geneious Prime and phylogenetic trees were constructed using maximum likelihood (ML) and Bayesian inference (BI) methods on the concatenated loci. ML analyses were conducted by RAxML v.8.2.11 (Randomized Accelerated Maximum Likelihood; Stamatakis 2014) implemented in Geneious Prime and MEGA11 (Tamura et al. 2021). Likelihood parameters followed the General Time Reversible model with a gamma distribution (GTR+G) and branch confidence of tree topology was assessed using 1,000 bootstrap replicates.

In RAxML, we used the '-f a -x 1' algorithm option and RAxML estimated all free parameters. In MEGA11, we used the default settings for likelihood parameters with partial deletion of gaps in the alignment. BI analysis of Posterior Probability (Pp) was conducted on the concate-

Table 1. Taxa included in the phylogenetic analysis with locality, catalogue number, and GenBank accession numbers. Newly-sequenced specimens are highlighted in bold. GMx, Gulf of Mexico; water body names preceded by N, S, E or W to indicate northern, southern, eastern, or western, respectively; –, no sequence identifier available.

Taxon name	Locality	Catalogue No.	GenBank Accession Nos.		
			12S	16S	ITS-1
Amphithrax aculeatus	W Atlantic, Barbados	**BLSZ 222**	**OR267308**	**OR267299**	**–**
	W Atlantic, Barbados	**BLSZ 223**	**OR267309**	**OR267300**	**–**
	W Atlantic, Barbados	**BLSZ 267**	**OR267311**	**OR267302**	**OR260473**
	W Atlantic, Barbados	**BLSZ 268**	**OR267312**	**OR267303**	**–**
	W Atlantic, Barbados	**BLSZ 252**	**OR267310**	**OR267301**	**OR260472**
Amphithrax armatus	E Pacific, Taiwan	NMMBCD 4083	–	MG281843	–
Amphithrax caboverdianus	E Atlantic, Cape Verde Island	ULLZ 11711	KF453086	KF452982	–
Amphithrax braziliensis	S Atlantic, Brazil	CCDB_BRA 5060	–	MF178237.1	–
Amphithrax hemphilli	Caribbean, Belize	ULLZ 9150	KF453133	KF453024	–
Amphithrax verrucosus	Caribbean, Panamá	ULLZ 13596	–	MK971519.1	–
	W Atlantic, Florida	ULLZ 4534	KF453096	KF452993	–
	Caribbean, Belize	ULLZ 9148	KF453131	KF453022	–
Amphithrax verrucosus	W Atlantic, Barbados	**BLSZ 226**	**OR267313**	**OR267304**	**–**
	W Atlantic, Barbados	**BLSZ 228**	**OR267134**	**OR267305**	**OR260474**
	W Atlantic, Barbados	**BLSZ 265**	**OR267316**	**OR267307**	**–**
	W Atlantic, Barbados	**BLSZ 250**	**OR267315**	**OR267306**	**OR260475**
Amphithrax verrucosus	W Atlantic, Venezuela	MOBR-C-1529	–	GQ438765	–
	W Atlantic, Venezuela	MOBR-C-1529	–	GQ438766	–
Hemus cristulipes	E GMx, Florida	ULLZ 5783	KF453100	KF452995	–
Hemus magalae	E Pacific, Panamá	USNM 1149374	KF453144	KF453034	–
Maguimithrax spinosissimus	Caribbean, Belize	ULLZ 6981	KF453130	KF453021	–
Mithraculus cinctimanus	Caribbean, Belize	ULLZ 12248	KF453091	KF452988	–
Mithraculus coryphe	Caribbean, Belize	ULLZ 9223	KF453135	KF453026	–
Mithraculus sculptus	W Atlantic, Florida	ULLZ 8774	GU144526	GU144539	–
Mithrax hispidus	W Atlantic, Florida	ULLZ 8619	GU144532	GU14450	–
Omalacantha antillensis	W Atlantic, Florida	ULLZ 5663	KF453099	KF452994	–
Omalacantha bicornutus	Caribbean, Belize	ULLZ 7077	KF453116	KF453008	–
Thoe puella	Caribbean, Colombia	ULLZ 9227	KF453136	KF453027	–
	W Atlantic, Florida	ULLZ 4533	KF453095	KF452992	–
Outgroup taxa					
Libinia emarginata	N GMx, Louisiana	ULLZ 10344	KF453078	KF452974	–
Maja brachydactyla	E Atlantic, Spain	ULLZ 11425	KF453082	KF452978	–

nated loci using MrBayes (plugin v.3.2.6: Huelsenbeck and Ronquist 2001) implemented in Geneious Prime. In MrBayes, the gamma category was set to four, with four heated MCMC of 1,100,000 generations, sampling every 200th tree and a burn-in of 10%; all other parameters remained free. ML and BI analysis were also performed on individual datasets of the 12S, 16S and ITS-1 genes (see supplementary material S1). The resulting best tree generated for the concatenated dataset was used to reflect phylogeny.

Results

Phylogenetic analysis

Our study generated new sequences for both *A. aculeatus* and *A. verrucosus* from specimens collected in Barbados. These sequences, along with sequences available from GenBank, were used to determine the taxonomic status of *A. verrucosus* and to establish its phylogenetic relationship with *A. aculeatus*. All three genes (12S rRNA, 16S rRNA and ITS-1) were successfully amplified and the phylogenetic analysis consisted of 31 terminals (including outgroup taxa, Table 1) that represents eight genera and 18 species. In total 1,408 base pairs (excluding primer regions) were aligned: 418 bp for 12S, 437 bp for 16S and 553 bp for ITS-1. Tree topologies were congruent with both Bayesian Inference (BI) and maximum Likelihood (ML) analyses and, as both ML and BI analyses resulted in similar topologies, only the BI tree is shown with ML bootstrap and BI support values are depicted on nodes (Fig. 2).

Amphithrax forms a monophyletic group with the Western Atlantic species *A. hemphilli* (Rathbun, 1892)

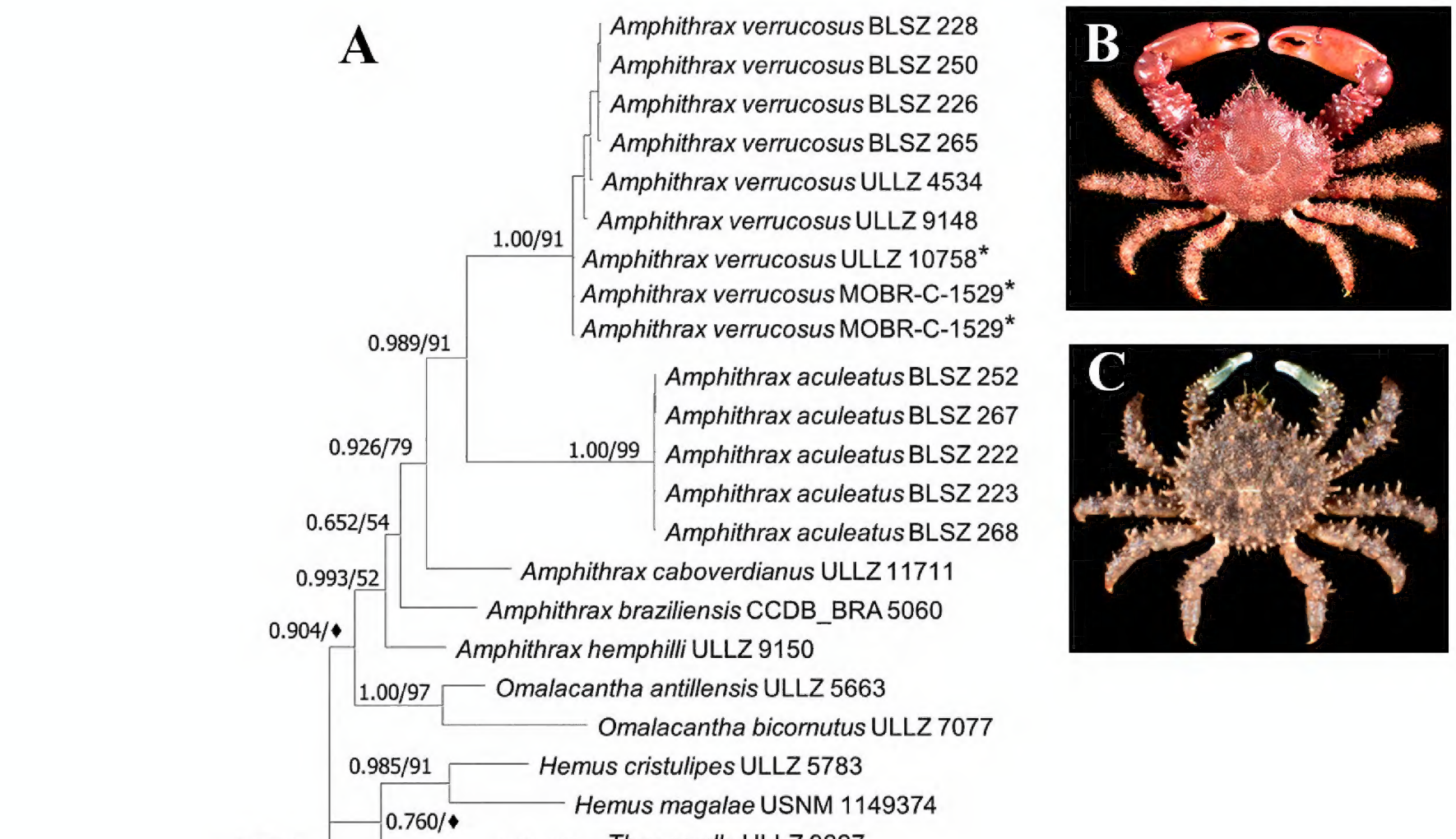

Figure 2. A. BI molecular phylogenetic tree for *Amphithrax verrucosus*, *A. aculeatus* and other selected species within the family Mithracidae MacLeay, 1838. Based on GTR+G nucleotide substitution model on the concatenated dataset for two mitochondrial (12S, 16S) and one nuclear (ITS-1) genes, represented as a maximum likelihood phylogram with Bayesian posterior probabilities and maximum likelihood bootstrap values (black diamond = ≤ 50% support, * = 16S sequences only). Note: ULLZ's 9148 and 4534, of Windsor and Felder (2014) and 13596 all re-identified as *Amphithrax verrucosus* (H. Milne Edwards, 1832); **B.** *Amphithrax verrucosus* (H. Milne Edwards, 1832), male (CW: 40.7 mm; CL: 29.5 mm), Barbados (BLSZ 218); **C.** *Amphithrax aculeatus* (Herbst, 1790), juvenile female (CW: 53.8 mm; CL: 44.0 mm), Barbados (BLSZ 217).

at the basal position. *Amphithrax verrucosus* is well supported (ML = 91%, Pp = 0.989) as a separate species and sister taxa to *A. aculeatus*. All specimens previously identified by Windsor and Felder (2014) as *A. aculeatus* (ULLZ 9148 and 4534) and an additional specimen from the ULLZ collection (ULLZ 13596) were reidentified as *A. verrucosus* based on the morphological examination and the sequences from Barbados (see supplementary material). The phylogenetic tree shows divergence of sequences from ULLZ (4534, 9148, 13596) and MO-BR-C-1529. *Omalacantha* Streets, 1871 is identified as a sister clade to *Amphithrax*, with a high BI support value (ML ≤ 50%, Pp = 0.904). This result is also congruent with that of Ng et al. (2018: fig. 5) where *Omalacantha* forms a sister clade to *Amphithrax*.

Our phylogenetic results show *A. armatus* forming a clade with *Maguimithrax* Klompmaker, Portell, Prueter & Tucker, 2015, and *Mithrax* Latreille, 1816, and not with *Amphithrax*, but the support for this relationship is very low [(ML ≤ 50%, Pp = 0.634) (Fig. 2)].

Systematics

Family Mithracidae MacLeay, 1838
Genus *Amphithrax* Windsor & Felder, 2017

Amphithrax aculeatus (Herbst, 1790)

Figs 2C, 3A–D, 5A, C, D, 6A–D

Cancer aculeatus Herbst, 1790: 248, pl. XVIII, fig. B, pl. XIX, fig. 104 [type-locality: Americas (probably between Florida and Venezuela); holotype (ZMB Herbst 0134) and paralectotype (ZMB Herbst 0079) in Berlin Museum]. — Desmarest (1825: 151).

Cancer spinipes Herbst, 1790: 241, pl. XVII, fig. 94 [type locality: probably West Indies; type: probably lost]. — Desmarest (1825: 151).

Mithrax aculeatus — H. Milne Edwards 1832: class 7; 1834–1840: 321; Schomburgk 1848: 65; Stimpson 1860: 188; Desbonne and Schramm 1867: 5; A. Milne-Edwards 1875: 102; Ng et al. 2008: 120; Windsor and Felder 2014: 163, figs 3G, 4H.

Mithrax pilosus Rathbun, 1892: 262, pl. 39 [type-locality: Abaco Island, Bahamas; holotype (USNM 16299) and paratypes (USNM 16299) in National Museum of Natural History, Washington, D.C.]. — Rathbun 1901: 66; 1921: 83; Nutting 1919: 75; Rodríguez 1980: 287; Abele and Kim 1986: 47, 522 fig. c; Wagner 1990: 25–29, figs 28–31; Carmona-Suárez and Poupin 2016: 373, fig. 6H.

Mithrax (*Mithrax*) *aculeatus* — Young, 1900: 90.

Mithrax (*Mithrax*) *pilosus* — Rathbun, 1925: 394, pl. 138, fig. 3; 1933: 29; Powers 1977: 57; Lira et al. 2013: 55, tab.1.

Amphithrax aculeatus — Windsor & Felder, 2017: 1; Poupin 2018: 192–193, fig. 210; Questel 2019: 16, unnumbered figure; Parasram et al. 2023: 20, fig. 9B.

Material examined. Type material. Paralectotype of *Cancer aculeatus* Herbst, 1790: Americas • dry carapace only, ZMB Herbst 79. Holotype of *Mithrax pilosus* Rathbun, 1982: Bahamas, Abaco Island, Albatross Research Vessel • ♂ (CW: 30.0; CL: 28.0 mm) (USNM 16299), 26°27'59.99"N, 77°04'60.00"W. Paratypes: Idem, 2 ♂♂, 1 juv. ♀ (CW: 29.0; 29.0; 19.0; mm; CL: 26.0; 26.0; 18.0 mm) (USNM 16299).

Additional material. Puerto Rico, Playa de Ponce, Lighthouse Reef • 1 ♂, 1 juv. ♂ (CW: 23.0; 9.0 mm; CL: 21.0; 9.0 mm) (USNM 24091), 18°00'39"N, 66°41'14"W. St. Croix, Christiansted Harbour • 1 ♀ (CW: 42 mm; CL: 35 mm) (USNM 72827), 17°44'47.9004"N, 64°42'11.5236"W. Virgin Islands, St. Croix, Buck Island, 1 ♂ (CW: 28 mm; CL: 25 mm) (USNM 73318), 17°47'14"N, 64°37'15"W. Guadeloupe, Anse Babin, KARUBENTHOS 2012 • 1 ♂ (MNHN-IU-2013-5929), 16°20'26.9988"N, 61°31'32.9988"W. Barbados • 1 ♂ (CW: 105.0 mm; CL: 87.0 mm) (USNM 1519303), 13°04'30.73"N, 59°36'12.49"W. Palm Court, St. Michael • 3 ♂♂, 1 juv. ♀ (CW: 10.0; 11.0; 11.4; 53.8 mm; CL: 11.0; 12.0; 44.0 mm) (BLSZ 217), nearshore rubble, 13°04'30.73"N, 59°36'12.49"W. Idem, 1 ♂, (CW: 37.0 mm; CL: 32.0 mm) (BLSZ 331). Idem, 1 juv. ♀, (CW: 34.4 mm; CL: 30.0 mm) (BLSZ 220). Idem, 1 juv. ♀, (CW: 33.4 mm; CL: 30.0 mm) (BLSZ 221). Idem, 1 ♂ (CW: 32.0 mm; CL: 28.0 mm) (BLSZ 222). Idem, 1 juv. ♀ (CW: 28.0 mm; CL: 24.0 mm) (BLSZ 223). Idem, 1 juv. ♀ (CW: 34.0 mm; CL: 29.0 mm) (BLSZ 267). Idem, 1 juv., ♀ (CW: 16.2 mm; CL: 16.0 mm) (BLSZ 268). Drill Hall, St. Michael • 1 juv. ♀, 1 ♂ (CW: 29.6; 24.7 mm; CL: 26.0, 21.0 mm) (BLSZ 120), nearshore rubble, 13°04'36.30"N, 59°36'26.63"W. Idem, 3 ♂♂, (CW: 12.4;12.5; 7.8 mm; CL: 13.0; 14.0; 9.6 mm) (BLSZ 129). Idem, 1 juv., ♀ (CW: 13.0 mm; CL: 13.3 mm) (BLSZ 252). Idem, 3 ♂♂, 1 juv., ♀ (CW: 22.5; 10.3; 10.4; 11.0 mm; CL: 20.0; 10.0; 10.0; 11.0 mm) (BLSZ 269). Schooner Bay, St. James • 2 ♂♂, 1 ♀ (CW: 10.6; 30.5; 40.5 mm; CL: not taken) (MZUSP 40878, 40884), nearshore rubble, 13°14'48.24"N, 59°38'40.90"W.

Diagnosis. Carapace with acute spines dorsally, mainly in gastric, branchial, cardiac regions. Third antennal segment with long spine distolaterally, distinctly visible dorsally. Chelipeds slender; carpus with strong, sharp spines. Palm of chela with 4–5 spines on the upper margin. Propodus of pereopods with two to four spines on the proximal half. Carapace olive green to brown in colour.

Remarks. Herbst (1790: 248) referred to *Cancer aculeatus* as a "very rare American crab", but *Amphithrax aculeatus* is very abundant in Barbados. It is possible that the Herbst assessment of the rarity of this species is due to sampling biases (methodology used and sampling time), which resulted in a small number of specimens collected and deposited to the Berlin Museum (Tavares and Mendonça 2022).

In the same work, Herbst (1790) described *Cancer spinipes*, and both its description and figure resemble *A. verrucosus*. However, Windsor and Felder (2014) selected the lectotype of *Cancer aculeatus* Herbst, 1790 as the neotype for *Cancer spinipes* Herbst, 1790, establishing these names as objective synonyms. Due to lack of material for *Cancer spinipes* Herbst, 1790, we have opted

to maintain its status as a synonym of *A. aculeatus*. This decision is based on the need for nomenclatural consistency in the absence of more definitive data. While Herbst (1790) did not specify any locality for *Cancer spinipes*, which he referred to as a "Chinese crab" in the description (Herbst 1790: 241 "Der Schild diefer chinesischen Krabbe ift herzförmig"), Desmarest (1825) cited both *Cancer spinipes* and *Cancer aculeatus* as originating from the East Indies. We believe that this is a labelling mistake (common at that time) given that both species are endemic to the Western Atlantic.

Amphithrax aculeatus can be separated from *A. verrucosus* by: (i) the carapace densely setose, forming a velvet-like cover in both juvenile and adult specimens (Figs 3A, B, 5C, 6 A–D) (vs. carapace almost without setae in fully developed specimens, few setae concentrated laterally; in juveniles, carapace interspace with long and short dark setae, not forming a velvet-like cover in *A. verrucosus*; Figs 4A, B, 5B, 6 E–H); (ii) anterolateral margins of the carapace with six spines, excluding the orbital spine, where the first, second and third anterolateral spines are accompanied by an accessory spine (Figs 3A, 6A, D) (vs. anterolateral and posterior margins of the carapace with six to eight spines, excluding the orbital spine, where the first and second anterolateral spine are accompanied by accessory spines in *A. verrucosus*; Figs 4A, 6E, G); (iii) third antennal segment with long spine distolaterally, distinctly visible dorsally (Fig. 3B) (vs. third antennal segment with short spine distolaterally, not visible dorsally in *A. verrucosus*; Fig. 4B); (iv) chelipeds slender in adults, carpus with several strong, acute spines and palm of chela with four or five spines on the upper margins (Figs 3A, 6A, C) (vs. chelipeds more massive, especially in adult males, carpus without spines dorsally, with one to three blunt teeth on the inner margin, and palm of chela smooth; Figs 4A, 6E, G); (v) propodus of pereopods with two to four spines on the proximal half (vs. propodus of pereopods without spines in *A. verrucosus*); (vi) third maxilliped covered with short setae (vs. third maxilliped without setae in *A. verrucosus*); (vii) dorsal surface of pleon in males and females are covered in small thick setae (vs. dorsal surface of pleon in males and females without setae in *A. verrucosus*; (viii) G1 long, slender, dorsoventrally flattened, with the proximal third slightly curved towards mesial line, lateral margin with pappose setae at the base; tip triangular with rounded mesial lobe (Fig. 3C, D) (vs. G1 long, robust, slightly dorsoventrally flattened, with the proximal third slightly curved towards mesial line, lateral margin with pappose setae from base to distal third of shaft, tip V-shaped, with fold just below tip in *A. verrucosus*; Fig. 4C, D; see also Wagner (1990) and Lianos et al. (2021); (ix) dorsal and ventral surfaces of live specimens are olive green to brown in colour with whitish fingers of chela (Figs 3A, 6A–C) (vs. dorsal surface of live specimens predominantly maroon in colour and mixed with white spots on ventral surface, in ventral view, only tips of fingers are white in *A. verrucosus*; Figs 4A, 6E–H). It is worth pointing out that Wagner's (1990: 27, fig. 30) illustration of an *Mithrax pilosus* G1 is that of a young specimen of *A. aculeatus*.

Ontogenetic intraspecific variations in *A. aculeatus* from juveniles to adults are: spines on the carapace, carpus, and pereiopods become larger and more acute, but can be broken in the larger individuals. In some juvenile specimens, the gastric region of the carapace has more sparse setae than other regions and the dorsal surface also has the presence of flattened granules, five spines on anterolateral margins, with small and blunt accessory spines. In contrast, adults have the dorsal and ventral surface of the cephalothorax covered in short tuft setae, which has the appearance of a velvet mat; the granules on dorsal surface of the carapace develop into spines; anterolateral margins with six spines that are accompanied with well-developed accessory spines that are spiniform in shape. The palm of chelipeds in juveniles can have one to four spines, while in adults, the palm has three to six spines.

Parasram et al. (2023) reported *Amphithrax aculeatus* being found in close association with *A. verrucosus*, *Mithraculus coryphe* (Herbst, 1801), *M. forceps* A. Milne-Edwards, 1875, *Eriphia gonagra* (Fabricius, 1781) and *Pachygrapsus transversus* (Gibbes, 1850). Specimens of *A. aculeatus* were collected by hand and with caged crab traps on the west and south coasts of Barbados. *Amphithrax aculeatus* females collected in Barbados are generally larger than males (see material examined). No sexually mature females were found.

Due to the recurrent misidentifications between *A. aculeatus* and *A. verrucosus* in literature, determining the geographic distribution for these species has proved to be challenging. Nevertheless, based on the morphological characteristics provided by some authors, it seems that *A. aculeatus* has a more restricted geographic range, which encompasses the insular Caribbean. For instance, most of the material examined by Rathbun (1925: 395, Fig. 5) (under the name *Mithrax pilosus*) was collected from Caribbean islands, except for two specimens (one male and one female) from Venezuela. The first mention of these specimens was made by von Martens (1872: 82), but this could be a labelling mistake since Caracas is not a coastal city. These specimens were deposited in the Berlin Museum. In addition, A. Milne-Edwards (1875: 103) also mentioned two specimens of *A. aculeatus* collected in Vera Cruz, Mexico by M. Brémond. Contacting both ZMB and MNHN was not successful in locating these materials, therefore, these specimens are herein classified under *A. verrucosus*, given the prevalence of this species in Central America.

As a result of Windsor and Felder's (2014) revision, multiple collections modified their taxonomic records to align with *A. aculeatus*, which resulted in the renaming of many specimens previously identified as *A. verrucosus* (see USNM and MNHN online catalogues). Based on our re-examination of the literature and materials

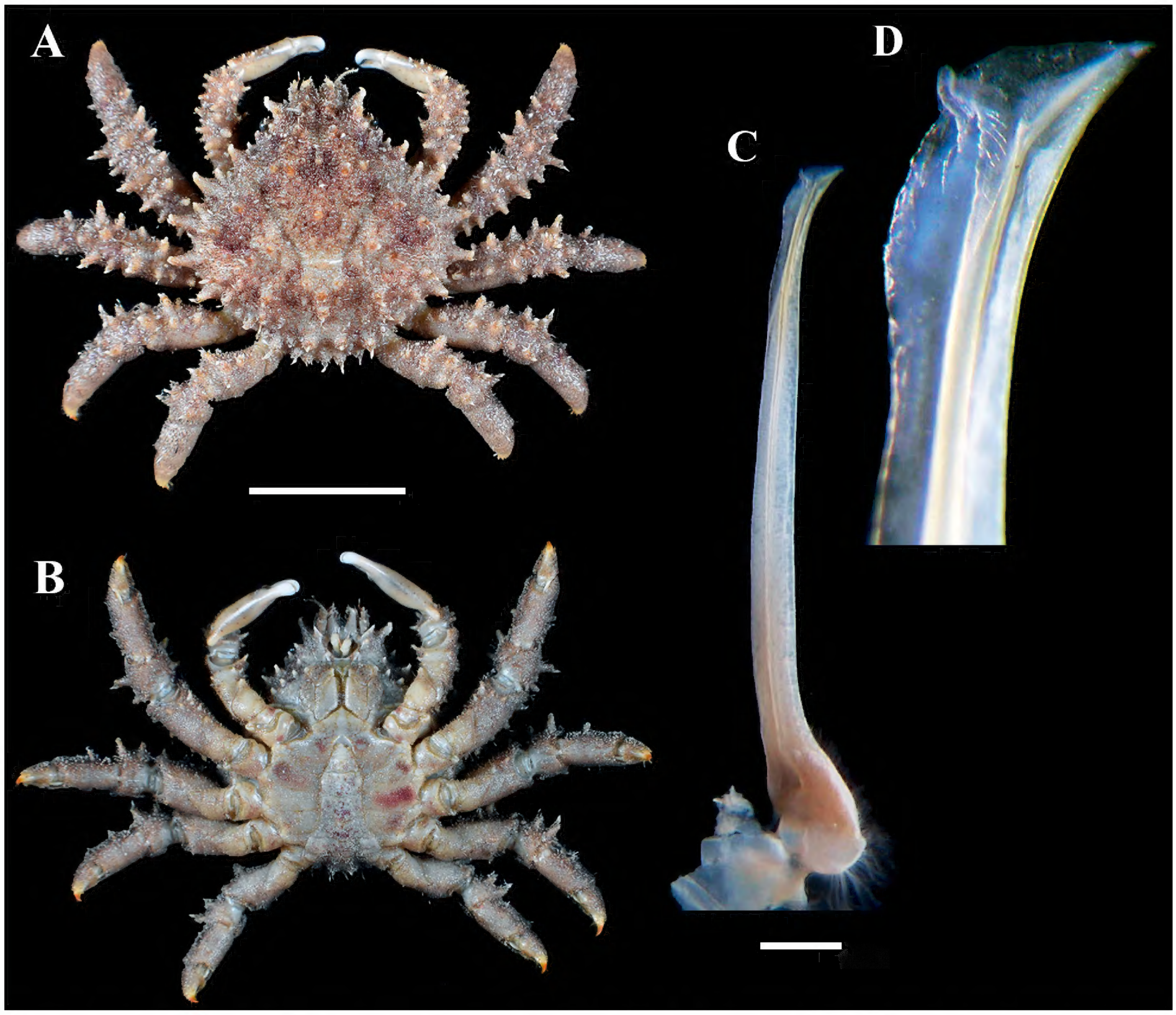

Figure 3. *Amphithrax aculeatus* (Herbst, 1790) adult male (BLSZ 331). **A.** Habitus, dorsal; **B.** Ventral view, locality: Barbados; **C.** Pleonal view of right G1 (BLSZ 222); **D.** Distal third of the right G1. Scale bars: 20 mm (**A, B**); 10 mm (**C**). Photos: Nadeshinie Parasram.

from different localities, we proposed the following update range for the two species: *A. aculeatus* is island endemic with a northern limit of Florida (Tortugas) and a southern limit of Barbados, whereas *A. verrucosus* has a northern limit of USA (South Carolina) and a southern limit of Brazil.

Colouration. In its natural habitat, the carapace of *A. aculeatus* exhibits an olive green to brown hue; but the carapace could be spotted with large beige spots (MNHN-IU-2013-5929) especially in younger individuals. The chelipeds are predominantly green (darker or lighter) with black spots on the palm, fingers are whitish (Figs 3A, 6 A–D). Pereopods are brown (light to dark) or green.

Distribution. Western Atlantic: USA (Florida, Dry Tortugas), Bahamas, Cuba, Puerto Rico, British Virgins Islands, St. Thomas, St. Croix, St. Martin, St. Barthélemy, Antigua, Guadeloupe, Martinique, and Barbados (Stimpson 1860; Rathbun 1925; Carmona-Suárez and Poupin 2016; Poupin 2018; present study).

Amphithrax verrucosus (H. Milne Edwards, 1832)

Figs 2B, 4A–D, 5B, 6E–H

Mithrax verrucosus H. Milne Edwards, 1832: 11, pl. 4 [type-locality: Martinique; type probably in MNHN]. — Schomburgk 1848: 65; Stimpson 1860: 187; Desbonne and Schramm 1867: 6; A. Milne-Edwards 1875: 102; Miers 1886: 86; Rathbun 1897: 9; 1921: 83; 1924: 20; Nutting 1919: 77; Rodriguez 1980: 287; Abele and Kim 1986: 47, 524 fig. c, d; Wagner 1990: 29–32, figs 32–35; Melo 1996: 239, unnumbered fig.; Ng et al. 2008: 120; Alves et al. 2012: 943, fig. 3C; Carmona-Suárez and Poupin 2016: 373, fig. 6I.

Mithrax verrucosus variety — Rathbun 1898: 259.

Mithrax (*Mithrax*) *verrucosus* — Young 1900: 93; Rathbun 1925: 400, pl. 144; 1933: 30; Boone 1927: 39; Williams 1965: 255, figs 235, 245B; 1984: 336, figs 271, 275c; Coelho and Ramos 1972: 215; Collins and Morris 1976: 119, pl. 17, fig. 7, pl. 18, figs 5–7; Powers 1977: 58; Keith 1985: 259, fig. 5E.

Mithrax plumosus Rathbun, 1901: 67 [type-locality: Puerto Real, Puerto Rico; holotype in USNM 23775].

Amphithrax aculeatus — Assugeni et al. 2017: 1630, tab. 1; Mantelatto et al. 2020: 39; Lianos et al. 2021: 1–19, figs 1, 11; Ortiz 2022: 34, fig. 14C (not *Cancer aculeatus* Herbst, 1790).

Amphithrax verrucosus — Poupin 2018: 193, fig. 211; Questel 2019: 16, unnumbered figure; Parasram et al. 2023: 21, fig. 9D.

Material examined. **Type material.** Holotype of *Mithrax plumosus* Rathbun, 1901: Puerto Rico, Port Real, Vieques, Fish Hawk Expedition • 1 ovig., ♀ (CW: 37.0 mm; CL: 29.0 mm) (USNM 23775), 18°7'29.8956"N, 65°26'31.6428"W.

Additional material. USA, Florida, Big Pine Key • 3 ♂♂, 3 ♀♀, 3 ovig. ♀♀ (USNM 14030), 24°40'11.514"N, 81°21'14.2884"W. Florida • 8 ♂♂, 3 ovig. ♀♀ (MCZ 353), 27°59'39.8472"N, 81°45'36.9144"W. Idem, 1 ♂, 1 juv. ♀ (MCZ unnumbered), 27°59'39.8472"N, 81°45'36.9144"W. Idem, 1 juv. ♀ (CW: 34.0 mm; CL: 20.0 mm) (ULLZ 4534), 27°59'39.8472"N, 81°45'36.9144"W. Idem, 1 ♂ (ULLZ 13956), 27°59'39.8472"N, 81°45'36.9144"W. Idem, Florida Keys • 1 ovig. ♀ (CW: 56.0 mm; CL: 42.0 mm) (ULLZ 15294), 24°41'29.0472"N, 81°11'22.8552"W. Idem, Big Pine Key • 1 ♂ (ULLZ 11924), 24°40'11.514"N, 81°21'14.2884"W. Idem, Loggerhead Key • 1 ♂ (CW: 67.0 mm; CL: 53.0 mm) (ULLZ 11736), 24°63'23.745"N, 82°92'06.767"W. Idem, Dry Tortugas • 1 ♂ (USNM 69068), 24°62'82"N, 82°87'32"W. Belize, Carrie Bow Cay Reef • 1 ♂ (CW: 10.0 mm; CL: 8.0 mm) (ULLZ 9148), 17°29'51.77"N,

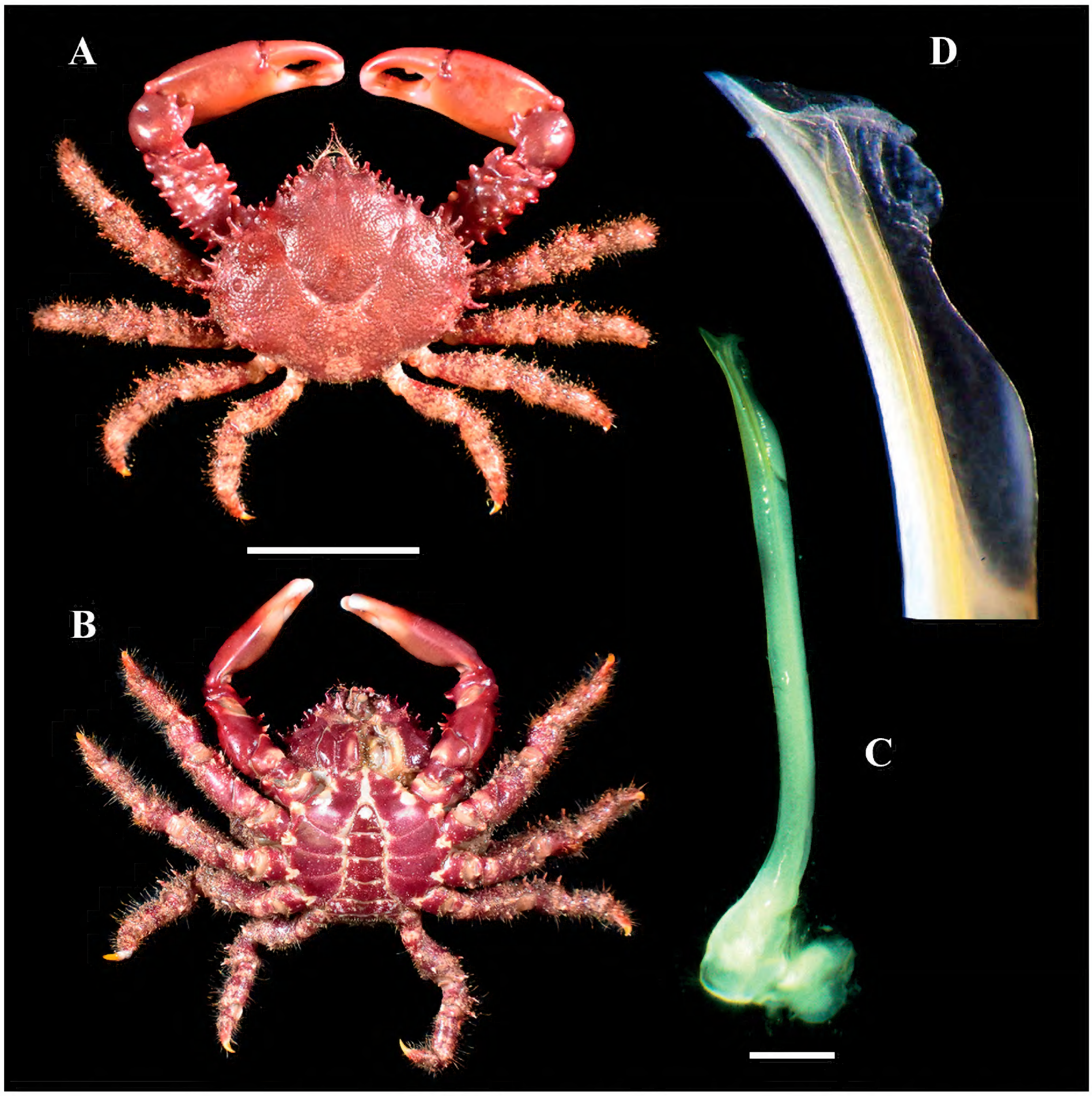

Figure 4. *Amphithrax verrucosus* (H. Milne Edwards, 1832) adult male (BLSZ 218). **A.** Habitus, dorsal; **B.** Ventral view, locality: Barbados; **C.** Pleonal view of left G1 (BLSZ 228); **D.** Distal third of left G1. Scale bars: 20 mm (**A, B**); 10 mm (**C**). Photos: Nadeshinie Parasram.

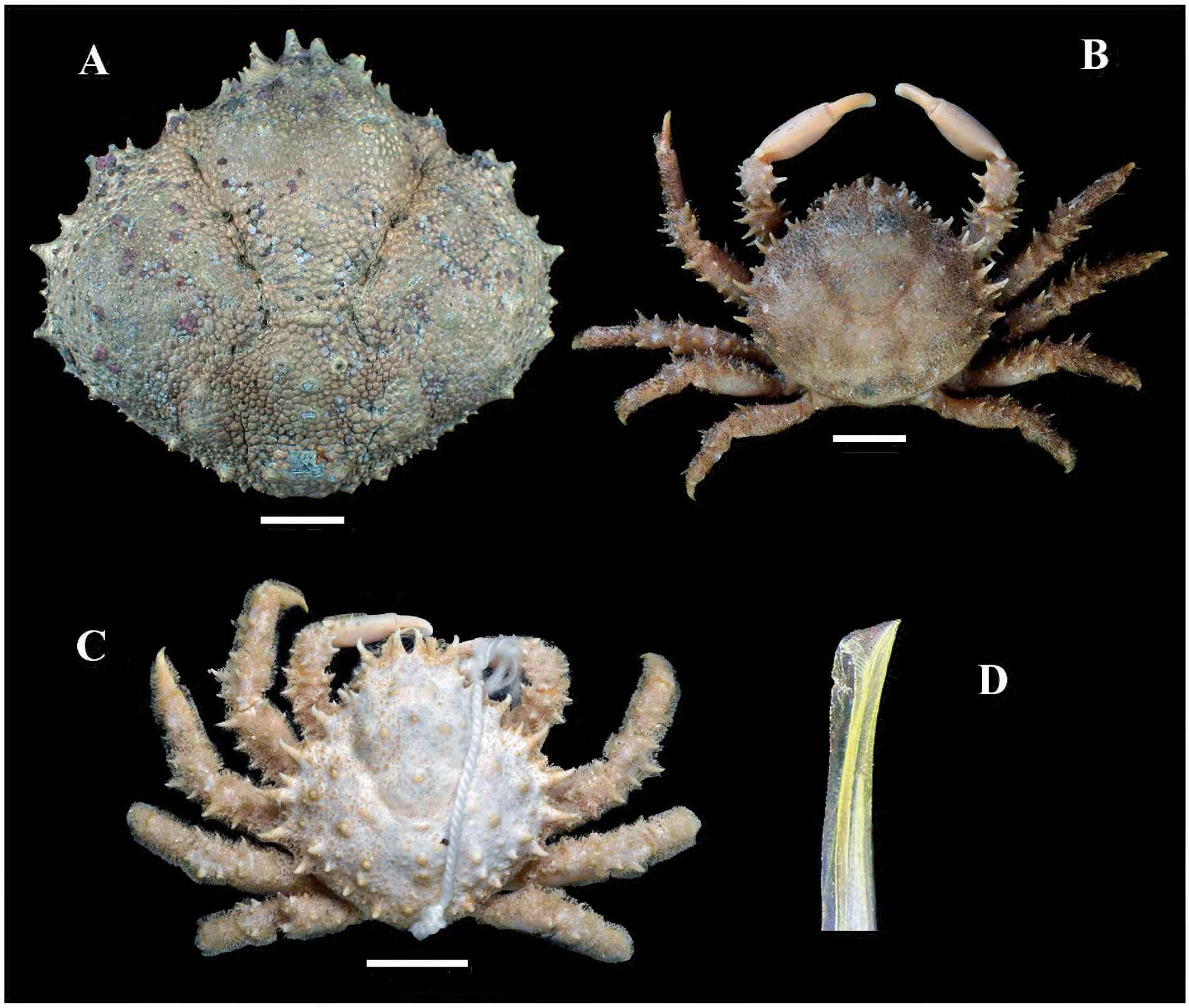

Figure 5. Type specimens. **A.** Paralectotype of *Cancer aculeatus*, Herbst, 1790, dry preserved carapace without setae and with most of the spines broken (ZMB Herbst 79), locality: Antilles; **B.** Holotype of *Mithrax plumosus* Rathbun, 1901 (USNM 23775), ovig. female (CW: 37.0 mm; CL: 29.0 mm), locality: Puerto Rico; **C.** Holotype of *Mithrax pilosus* Rathbun, 1892, setae on carapace removed (USNM 16299), male (CW: 30.0 mm; CL: 28.0 mm), locality: Bahamas; **D.** Distal third of the right G1 of the holotype of *Mithrax pilosus* (USNM 16299) in pleonal view. Photos: **A** Kristina von Rintelen. **B, C, D** Amanda Windsor.

88°11'11.95"W. Honduras, Swan Island • 1 ♂ (USNM 15074). Guadeloupe, Pointe Baham, MADIBENTHOS • 1 ♂ (MNHN-IU-2016-10018), 14°24'42.9984"N, 60°50'4.9956"W. Martinique, Point Jacob, MADIBENTHOS • 1 ♀ (MNHN-IU-2017-10364), 14°36'8.0028"N, 60°48'57.996"W. Barbados, Palm Court, St. Michael • 1 ♀ (CW: 31.6 mm; CL: 24.0 mm) (BLSZ 225). Idem, 1 ovig. ♀ (CW: 26.2 mm; CL: 22.0 mm) (BLSZ 227), nearshore rubble, 13°04'30.73"N, 59°36'12.49"W. Idem, 1 ♂ (CW: 26.7 mm; CL: 22.0 mm) (BLSZ 228). Idem, 1 ♀ (CW: 31.4 mm; CL: 24.5 mm) (BLSZ 328). Idem, 1 juv. ♀ (CW: 12.4 mm; CL: 12.0 mm) (BLSZ 332). Idem, 1 ♂ (CW: 24.5 mm; CL: 23.0 mm) (BLSZ 229). Idem, 1 ♀ (CW: 30.2 mm; CL: 25.6 mm) (BLSZ 251). Idem, 1 ♂ (CW: 21.1 mm; CL: 18.2 mm) (BLSZ 226). Drill Hall, St. Michael • 3 ♀, 1 ♂ (CW: 27.1; 11.3; 5.3 mm; CL: 22.0; 12.5; 6.0) (BLSZ 123), nearshore rubble, 13°04'36.30"N, 59°36'26.63"W. Idem, 1 ♀ ovig., (CW: 26.4 mm; CL: 27.5 mm) (BLSZ 266). Idem, 1 ♂ (CW: 27.2 mm; CL: 22.0 mm) (BLSZ 265). Idem, 1 ♂ (CW: 35.1 mm; CL: 27.4 mm) (BLSZ 250). Idem, 1 ovig., ♀ (CW: 31.6 mm; CL: 25.7 mm) (BLSZ 249). Idem, 12 ♂♂, 3 ♀♀, 2 juv., ♀♀ (CW: 7.5 mm; 14.3 mm; 16.6 mm; 22.0 mm; 14.2 mm; 13.6 mm; 23.5 mm; 8.5 mm; 22.0 mm; 15.2 mm; 15.0 mm; 20.3 mm; 24.0 mm; 25.7 mm; 17.4 mm; 20.0 mm; 15.0 mm; CL: 7.0 mm; 13.1 mm; 15.0 mm; 18.4 mm; 12.6 mm; 12.7 mm; 19.1 mm; 7.0 mm; 19.0 mm; 14.8 mm; 14.0 mm; 17.0 mm; 20.0 mm; 21.0 mm; 20.5 mm; 17.0 mm;13.6 mm) (BLSZ 278). Batts Rock, St. Michael • 1 ♂ (CW: 26.7 mm; CL: 21.5 mm) (BLSZ 165) subtidal, 13°08'04.81"N, 59°38'12.30"W. Idem, 1 ♂ (CW: 40.5 mm; CL not taken) (MZUSP 40921). Coconut Court, Christ Church • 2 ♂♂, 1 ♀, 1 ovig., ♀ (CW: 16.7 mm; 6.0 mm; 15.5 mm; 20.0 mm; CL: 15.5 mm; 6.4 mm; 14.7 mm; 22.8 mm) (BLSZ 211), nearshore rubble, 13°04'31.59"N, 59°36'13.78"W. Trinidad and Tobago, Chacachacare, Rusts Bay • 1 ♂ (CW: 42.0 mm; CL: 33.0 mm) (USNM

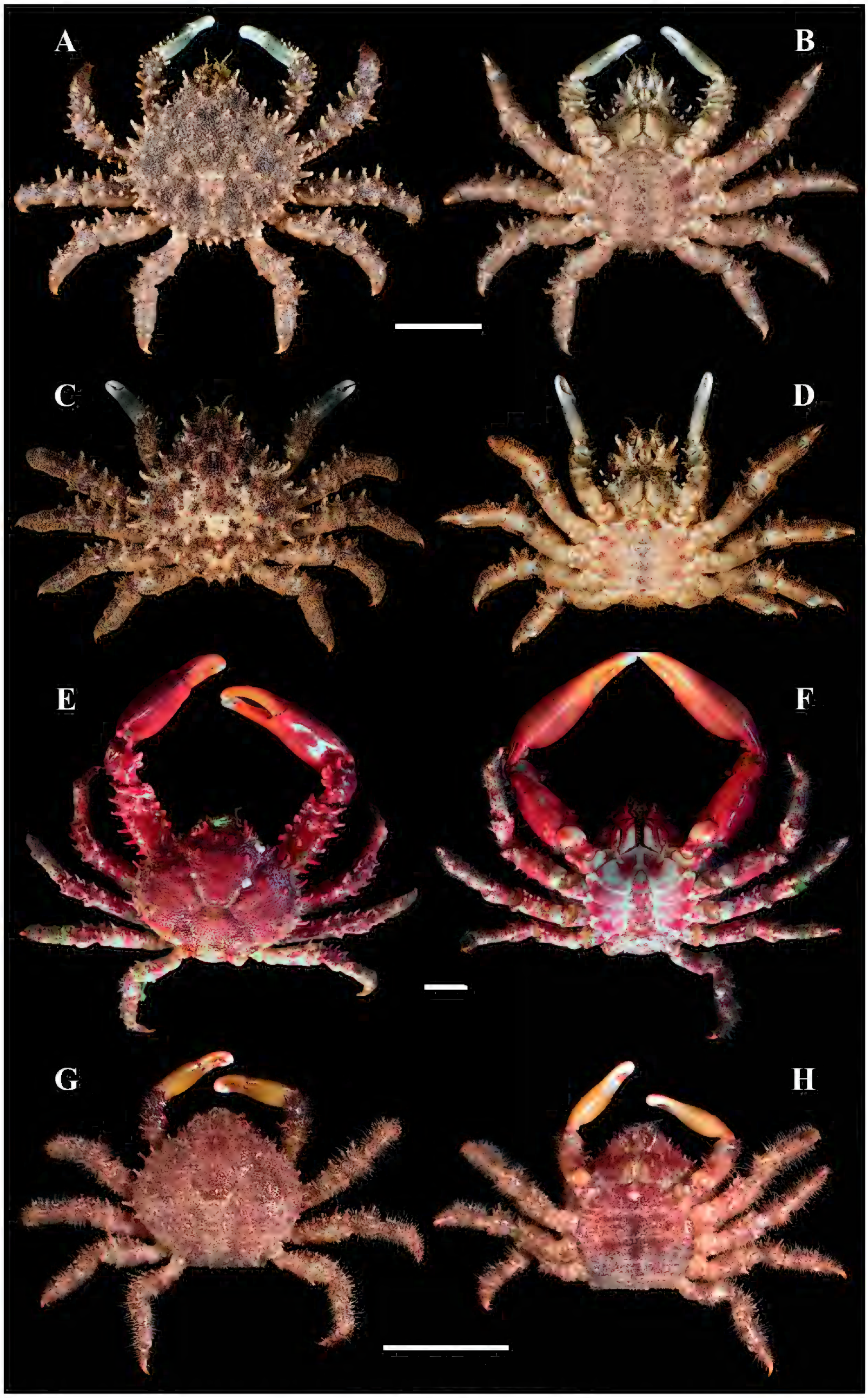

Figure 6. Colour in life. Young female of *Amphithrax aculeatus* (Herbst, 1790) (BLSZ 217), Barbados. **A.** Habitus dorsal view; **B.** Ventral view. Adult male of *Amphithrax aculeatus* (Herbst, 1790) (MNHN-IU-2013-5929), Guadeloupe; **C.** Habitus, dorsal view; **D.** Ventral view. Adult male of *Amphithrax verrucosus* (H. Milne Edwards, 1832) (GIC 072), Venezuela; **E.** Habitus, dorsal view; **F.** Ventral view. Female of *Amphithrax verrucosus* (H. Milne Edwards, 1832) (BLSZ 328), Barbados; **G.** Habitus, dorsal view; **H.** Ventral view. Scale bars: 20 mm. Photos: **A, B, G, H.** Nadeshinie Parasram. **C, D.** Joseph Poupin. **E, F.** William Santana.

137759), 10°40'59.99"N, 61°44'59.99"W. Venezuela, Isla de Coche, Bajo Culebra • 2 ♀♀, 3 ♂♂ (GIC 072), 10°46'21.59"N, 63°56'24.59"W. Idem, 1 ♂ (LSZ 012), 10°46'21.59"N, 63°56'24.59"W. Isla de Cubagua • 1 ♂, 1 ♀ (LSZ 012), 10°49'4.79"N, 64°10'34.20"W. Brazil, Rio Grande do Norte, Atoll das Rocas • 1 ♂ (MZUSP 15323), 3°51'59.99"N, 33°48'59.99"W. Idem, 1 ♂ (MZUSP 24761), 3°51'59.99"N, 33°48'59.99"W. Idem, 2 ♂ (MNRJ 15609), 3°51'59.99"N, 33°48'59.99"W. Idem, 3 ♂♂, 1 ovig., ♀ (MNRJ 4782), 3°51'59.99"N, 33°48'59.99"W. São Paulo, Ilha Vitória • 3 ♂♂ (MZUSP 16708), 20°19'9.98"N, 40°20'16.01"W.

Diagnosis. Dorsal surface of carapace uniformly covered with closely-set granules of irregular size; there are a few blunt tubercles on the gastric, branchial and cardiac regions; spines are only present in the lateral margins. In fully developed males chelipeds massive, carpus dorsally smooth with few blunt, short tubercles dorsolaterally in some specimens, strong teeth on inner margins and the palm of the cheliped is smooth. Propodi of pereopods are without spines, but have the presence of long and short dark setae. Carapace is predominantly maroon in colour.

Remarks. Much of the ambiguity surrounding *A. aculeatus* and *A. verrucosus* originated when Rathbun described *Mithrax pilosus* and *M. plumosus* (Rathbun 1892, 1901; Fig. 5B, C). These descriptions were based on small specimens, which naturally appear more similar than their fully matured counterparts. Additionally, Windsor and Felder (2014) based their decision on very small juvenile specimens, mistakenly identified as *A. aculeatus* (see Suppl. material 2 in supplementary material and phylogenetic section of this work). Despite the similarities observed in younger specimens, the distinct morphological characteristics presented should suffice to prevent any further confusion between the two species.

The G1 of *A. verrucosus* was described in detail by Lianos et al. (2021) under the name *A. aculeatus* and by Wagner (1990) as *Mithrax verrucosus* (see also remark section for *A. aculeatus*).

Ontogenetic intraspecific variations in *A. verrucosus* from juveniles to adults are: juvenile specimens have short and long setae that are evenly distributed on the carapace, cheliped and third maxillipeds, while adults have much less setae or are devoid of setae in these regions. Some very small specimens may have few spines on the carapace, especially on branchial regions. Anterolateral margins with six to eight spines on juveniles, whereas adults have eight spines. Younger specimens and females with strong short spines dorsally on the carpus of the cheliped, which are not present in fully developed adults, remaining only inner carpal spines. The carapace spines on the branchial region, along with the spines dorsally on carpus in young specimens may have led some authors to confuse *A. verrucosus* with *A. aculeatus*. It is important to note that even in very small specimens, the palm of the cheliped and the propodus of pereopods in *A. verrucosus* lack spines. *Amphithrax verrucosus* is very abundant in Barbados as well and specimens were collected by hand and with caged crab traps on the west and south coasts of Barbados. Parasram et al. (2023), reported *Amphithrax verrucosus* as being found in close association with *A. aculeatus*, *Mithraculus coryphe* (Herbst, 1801), *Mithraculus forceps* A. Milne-Edwards, 1875, *Eriphia gonagra* (Fabricius, 1781) and *Pachygrapsus transversus* (Gibbes, 1850). Females of this species are generally smaller than males (see materials examined). Ovigerous females were collected in June and September 2021.

Colouration. Colour in life for *A. verrucosus* is carapace predominantly maroon and maroon mixed with white spots on ventral surface. Some specimens are more brownish, with light spots on the carapace (MNHN-IU-2016-10018) and some adult females have a light brown carapace (MNHN-IU-2017-10364). The chelipeds range from a dark maroon, greenish-brown to a lighter brown hue; in ventral view, only the tip of the fingers is whitish (Figs 4A, 6 E–H) (see also fig. 3G of Windsor and Felder (2014).

Distribution. Western Atlantic: USA (South Carolina to Florida), Gulf of Mexico, Cuba, Jamaica, Puerto Rico, Virgin Islands, St. Maarten, Honduras, Antigua, Belize, Guadeloupe, Isla de Aves, Martinique, Barbados, Grenada, Trinidad and Tobago, Bonaire, Curaçao, Aruba, Colombia, Venezuela, and Brazil, from Fernando de Noronha to São Paulo (Rathbun 1925; Carmona-Suárez and Poupin 2016; Poupin 2018; present study).

Discussion

Windsor and Felder (2014) recommended that *A. verrucosus* should be regarded as a junior subjective synonym of *A. aculeatus* citing substantial intraspecific morphological variation as the basis for their assertion. However, their comparisons were constrained by the limited number of specimens, all misidentified and they heavily relied on the original illustration from Herbst (1790). Upon thorough examination of comprehensive comparative materials, the morphological and molecular data detailed herein advocates for the recognition of *Amphithrax aculeatus* and *A. verrucosus* as distinct species.

Wagner (1990) was the first to study the G1 of *A. aculeatus* (as *Mithrax pilosus*) and *A. verrucosus* (as *Mithrax verrucosus*) showing the differences between both species. Our findings align with Wagner's (1990) observations. On the other hand, Windsor and Felder (2014) overlooked these nuanced disparities when synonymising the two species. This oversight may shed light on the subsequent conflation of *A. verrucosus* with *A. aculeatus*, especially given the pronounced differentiation in the G1 of each species.

Specimens of *A. aculeatus* are a close match to the plate of *Cancer aculeatus* by Herbst (1790) and *Mithrax pilosus* by Rathbun (1892) (Figs 3, 5A, C, D), whereas specimens of *A. verrucosus* resemble the plate of *Mithrax verrucosus* by H. Milne Edwards (1832) and *Mithrax plumosus* by Rathbun (1901) (Figs 4, 5B). Windsor and Felder's (2014: figs 3G, 4H) images of habitus and third maxilliped of a presumed *A. aculeatus* specimen are, in fact, a specimen of *A. verrucosus* (see Figs 4A, D, 6E–H in the present work).

Although the phylogenetic analysis performed supports the monophyly of the genus *Amphithrax*, the ML shows low support for this topology in contrast with the BI analysis (ML = 52%, Pp = 0.99). Nevertheless, the molecular separation of *A. aculeatus* and *A. verrucosus* as two different species and sister taxa is well supported and, in our phylogenetic tree, both species occupy distinct phylogenetic clusters (Fig. 2). Additionally, the divergence observed in ULLZ 13596 and MOBR-C-1529 can be attributed solely to the presence of 16S sequence alone in the dataset, no 12S or ITS-1 sequences being available for these specimens. A similar scenario is observed for ULLZ 4534 and 9148, where the divergence of these sequences in the phylogenetic tree is solely due to 12S and 16S sequences in the dataset. Our study presents the first molecular data for *A. aculeatus* and, additionally, we also provide ITS-1 sequences for *A. verrucosus*, thereby contributing valuable molecular insights for both species. The phylogenetic analysis of Windsor and Felder (2014) contained sequence data from *A. verrucosus* (see Suppl. material 2), which they misidentified as *A. aculeatus*. Our phylogenetic analysis was based on sequence data from both *A. aculeatus* and *A. verrucosus*. The inclusion of both species in our in our study produced different results than those obtained by Windsor and Felder (2014). *Amphithrax* in its majority is an Amphiamerican genus, with only *A. caboverdianus* found exclusively in the eastern Atlantic. The phylogenetic results are consistent with the conclusions drawn by Windsor and Felder (2014) and Ng et al. (2018) with *A. caboverdianus* occupying a basal position to *A. aculeatus* and *A. verrucosus* with relatively high support (ML = 79%, Pp = 0.926).

The phylogenetic analysis suggests that the taxonomic assignment of *Amphithrax armatus* within the *Amphithrax* is questionable. *Amphithrax armatus* aligns with species of *Maguimithrax* and *Mithrax*; however, the support for this relationship is low. Ng et al. (2018) also questioned the placement of *A. armatus* in *Amphithrax*, based on their morphological and phylogenetic analysis, which shows a close phylogenetic relationship of *A. armatus* to *Ala* Lockington, 1877 and *Nonala* Windsor & Felder, 2014. Nevertheless, the overall morphology of the carapace, third maxilliped and G1 of species within *Ala* and *Nonala* are very different when compared to *A. armatus*. Given that our phylogenetic analysis encompasses only sequences from 12S and 16S and lacks representatives from *Ala* and *Nonala*, this might account for the discrepancies observed between our study and Ng et al. (2018) results.

Windsor and Felder (2014) commented on the wide degree of morphological variations seen in *Amphithrax* and mentioned the possible removal of some species of *Amphithrax*. Although our study indicates that *A. armatus* may not belong to *Amphithrax*, we have opted to retain its classification for the time being as suggested by Ng et al. (2018). It is important to note that our study did not encompass a comprehensive revision of the entire *Amphithrax*. Therefore, to address these questions definitively, further-in-depth analysis of all species of *Amphithrax* incorporating both molecular and morphological evidence, as indicated by our results, is necessary. These suggestions were also mentioned by Windsor and Felder (2014) and Ng et al. (2018).

Conclusions

Our study underscores the importance of employing an approach that incorporates both morphology and molecular analysis in biodiversity research. We also emphasise the importance of utilising morphological characters such as the G1 in Brachyura for species identification in taxonomic studies.

Based on the evidence provided, we proposed a taxonomic revaluation, elevating *A. verrucosus* from junior subjective synonym to valid species status. This would bring the total number of species in the genus *Amphithrax*, *sensu stricto* to 11: *A. aculeatus*, *A. armatus*, *A. bellii* (Gerstaecker, 1857), *A. besnardi* (Melo, 1990), *A. braziliensis* (Rathbun, 1892), *A. caboverdianus*, *A. clarionensis* (Garth, 1940), *A. hemphilli* (Rathbun, 1892), *Amphithrax leucomelas* (Desbonne, in Desbonne & Schramm, 1867), *A. tuberculatus* (Stimpson, 1860), and *A. verrucosus* (H. Milne Edwards, 1832).

Additionally, the information provided in our study contributes to the existing body of data (morphological and molecular) that can be valuable for future taxonomic investigations within the superfamily Majoidea and biodiversity research in Caribbean Island hotspots.

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Supplementary material 1

Phylogenetic trees of individual datasets of 12S, 16S, and ITS-1 genes

Authors: Nadeshinie Parasram, William Santana, Yvonne Vallès, Amanda Windsor, Henri Vallès

Data type: tif

Explanation note: ML reconstruction for individual datasets of 12S, 16S and ITS-1 genes, represented as a maximum likelihood phylogram with maximum likelihood bootstrap values and Bayesian posterior probability (black diamond = ≤ 50% support).

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Link: https://doi.org/10.3897/zse.100.109192.suppl1

Supplementary material 2

Specimens used in the molecular analysis of Windsor and Felder (2014), which were erroneously identified as *Amphithrax aculeatus* (Herbst, 1790)

Authors: Nadeshinie Parasram, William Santana, Yvonne Vallès, Amanda M. Windsor, Henri Vallès

Data type: tif

Explanation note: Habitus, dorsal view of carapace of *Amphithrax verrucosus* (H. Milne Edwards, 1832). A ULLZ 4534, juvenile female (CW: 34.0 mm; CL: 20.0 mm), locality: Florida Keys; B ULLZ 9148, juvenile male (CW: 10.0 mm; CL: 8.0 mm), locality: Belize; C ULLZ 10758, juvenile female (CW: 15.0 mm; CL: 13.0 mm), locality: Panama. Scale bar: 20 mm. Photos: Amanda Windsor.

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